
**The Role of Circadian Rhythms
in Reproduction:
Development and Fertility in the *bmal1*
Null Mouse**

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fulfilment of the requirements for admission to the
degree of Doctor of Philosophy (PhD)

Declaration

This thesis contains no material which has been accepted for any other degree or diploma at any other university, nor to the best of my knowledge, does it contain any previously published material by another person, except where due reference has been made in the text of this thesis.

I consent to this thesis being made available for photocopying and loan if accepted for the award of the degree.

Michael James Boden

Signed:.....

Friday 21st April 2008

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Abbreviations

ARNT	Aryl-hydrocarbon Receptor Nuclear Transferase
bHLH	basic Helix-Loop-Helix binding domain
BMAL1	Brain Muscle ARNT-Like protein 1, Mop3, Arntl, JAP3, ARNT3,
Clock	Circadian Locomotor Output Cycles Kaput
Cry	Cryptochrome
CT	Circadian Time – time from expected onset of “lights on”
DBP	albumin D element-Binding Protein
DEC1	Differentially Expressed in Chondrocytes 1, Stra13, Sharp-2
DEC2	Differentially Expressed in Chondrocytes 2, Sharp-1
dNTP	deoxynucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
ETOH	Ethanol
hCG	Human Chorionic Gonadotropin
HSD3b1	3b-hydroxysteroid dehydrogenase, 3 β -HSD
IGF	Insulin-like Growth Factor
IU	International Units
KO	Knockout
Npas2	Neuronal PAS domain protein 2
P450scc	P450 side chain cleavage enzyme, Cyp 11a1, cytochrome P450 11a1
PBS	Phosphate Buffered Saline
Per	Period
PMSG	Pregnant Mares Serum Gonadotropin
POMA	Magnocellular pre-optic nucleus
Rcf	Relative Centrifugal Force
RORA	Retinoic-acid-receptor-related Orphan Receptor A
RORE	Retinoic-acid-receptor-related Orphan receptor A Response Element
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S.C.	Sub Cutaneous
StAR	Steroidogenic Acute Regulatory protein

TBE	Tris-Boric Acid-EDTA Buffer
Tris	Trishydroxymethylaminomethane, or 2-amino-2-hydroxymethyl-1,3-propanediol
VIA	Video Image Analysis
WT	Wild Type
ZT	Zeitgeber Time – time from the onset of “lights on”

Abstract

Circadian rhythms are the endogenous cycling of hormones, activity patterns and gene expression that occur across 24 hours. Disruption of circadian rhythms has been associated with multiple health complications, including reduction of fertility. The *bmal1* mouse provides an animal model for central and peripheral loss of rhythmicity. Herein the reproductive function and postnatal development in the *bmal1* knockout mouse has been evaluated.

The reproductive capability of the heterozygous breeding colony was investigated, with around 50% of the female breeder mice becoming pregnant within one estrus cycle. The offspring of the colony had a higher than expected level of perinatal mortality while the knockout and heterozygous genotype was under represented among the offspring surviving to weaning, suggesting high knockout embryo or perinatal losses. The circadian phenotype of this mouse model was confirmed, identifying the severe disruption of circadian behavioural rhythms. Further, the growth of the *bmal1* knockout mice was retarded compared with their heterozygous and wild type littermates from weaning to 9 months of age.

The reproductive function of the homozygous male *bmal1* knockout mouse was evaluated. They showed poor fertility, poorly developed secondary sexual organs, reduced sperm count and reduced sperm motility. Female *bmal1* knockout mice had delayed vaginal opening, delayed onset of first estrus, disrupted estrus cyclicity as well as impaired reproductive and mammary tissue development. Steroid hormone synthesis was compromised in both males (testosterone) and females (progesterone) and ovarian morphology revealed reduced corpora lutea formation and structural abnormalities. Female *bmal1* knockout mice also evidenced profound infertility, which was caused by a continuum of reproductive insufficiencies including reduced ovulation of oocytes, poorer progression of the preimplantation embryo and failure to successfully implant in the uterus. While the ovaries of *bmal1* knockout females were able to respond to exogenous stimulation, the number of ovulated oocytes was reduced, the fertilised oocytes were of reduced

quality, progressed poorly to mature blastocyst and once again failed to implant.

A bioinformatical evaluation of a panel of genes closely involved in reproduction and ovarian function was analysed for the presence of circadian enhancer regions (E-box sequences) or RORA response elements (RRE) in their promoter regions. It was revealed that many of the genes investigated contained one or more circadian E-box and RRE sequence, providing a mechanism for the disruption of circadian gene expression within the ovary to cause detrimental changes in gene expression. Further to this, the gene expression profile of these functional genes and clock genes were evaluated in ovarian tissues from wild type and knockout mice across the estrus cycles and across 24 hours. It was shown that the murine ovary rhythmically expressed the genes involved with the molecular clock across 24 hours, as well as several other genes previously associated with rhythmicity in peripheral tissues. Further, the loss of functional *bmal1* gene expression resulted in up or down regulation of over 75% of the functional genes investigated, including *steroidogenic acute regulatory protein* (the rate limiting enzyme for progesterone synthesis).

In conclusion, the *bmal1* knockout mouse shows a significant multi-factorial loss in fertility in both males and females. This loss occurs across a range of tissues and results in heavily reduced fertility in the male and complete infertility in the female. Further research could identify in greater detail the precise molecular mechanisms underpinning of this disruption.